Longitudinal Modeling of Adiposity in Periadolescent Greek Schoolchildren

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ABSTRACT

KOUTEDAKIS, Y., C. BOUZIOTAS, A. D. FLOURIS, and P. N. NELSON. Longitudinal Modeling of Adiposity in Periadolescent Greek Schoolchildren. *Med. Sci. Sports Exerc.*, Vol. 37, No. 12, pp. 2070–2074, 2005. **Purpose:** Obesity has an etiology that is multidimensional in nature. Given the dearth of longitudinal data, we examined changes in adipose tissue (Ad) in relation to physical activity levels (PA), aerobic fitness (AF), and energy intake (EI) in Greek schoolchildren, as they progressed from age 12 to 14 yr. **Methods:** This was a 2-yr and three–time-point (TP) study. Participants (N = 210 (TP_1); = 204 (TP_2); = 198 (TP_3)) were assessed for anthropometry, maturity status, Ad, PA, AF, and EI. Mean values were used for exploratory analyses, whereas two generalized estimating equations (GEE) models examined for longitudinal associations between the studied parameters. The first (GEE_1) aimed to extract inherent associations between the dependent (Ad) and independent (PA, AF, EI) variables for the entire study period. For further evidence of association, the second analysis (GEE_2) used the independent variables at TP_1 and TP_2 to predict the dependent variables at TP_3. **Results:** Levels of Ad in boys decreased significantly ($P < 0.05$) from TP_1 to TP_3, whereas the same variable demonstrated a nonsignificant increase ($P > 0.05$) in girls. GEE_1 revealed that longitudinal changes in Ad were significantly associated only with PA ($\beta = -0.16$; $P < 0.001$) and AF ($\beta = -0.09$; $P < 0.05$) for all schoolchildren. Similarly, GEE_2 revealed that the main factors (at TP_1 and TP_2) predicting the development of Ad (at TP_3) were PA ($\beta = -0.14$; $P < 0.001$) followed by AF ($\beta = -0.10$; $P < 0.05$). **Conclusion:** With respect to data presented, we established that longitudinal changes in Ad are mainly accompanied by changes in PA and, to a lesser extent, AF levels. **Key Words:** BODY FAT, PHYSICAL ACTIVITY, AEROBIC FITNESS, ENERGY INTAKE

Obesity is a major global epidemic involving both children and adults. In adults, ample epidemiological evidence demonstrates a positive association between obesity and a greater risk of all-cause mortality, particularly from coronary heart disease (CHD) (30). Similarly, overweight or obese children are more likely to display aberrant CHD risk factor profiles (5), develop a distorted self-image that appears to persist into adulthood (29), and are at increased risk for becoming overweight or obese adults (24). The etiology of obesity in childhood, therefore, is important to the study and implications of obesity in later life.

Although obesity has a strong genetic background (11), environmental and lifestyle factors are commonly considered the underlying causes of the condition. Cross-sectional studies have shown obese children to be less active, have lower aerobic fitness (AF), and demonstrate different eating patterns than their nonobese counterparts (19,25). Although longitudinal studies can provide a more extensive insight into the association between different variables, relatively few sets of pediatric longitudinal data have examined changes in childhood fatness in relation to lifestyle parameters with conflicting outcomes (9,12,22). Hence, a need exists for more such studies based on different sociocultural and environmental settings.

Greek children demonstrate increased prevalence of obesity (15), decreased physical activity (PA) levels (18), and, compared with their European and North American counterparts, low AF levels (14). Although research indicates an alarmingly high prevalence of pediatric obesity among Greek children, to our knowledge, a paucity of longitudinal epidemiologic data examine associations between selected lifestyle parameters and changes in adiposity during the early or midpuberty years. In view of the dearth of such data, the present study was conducted to investigate changes in adipose tissue (Ad) in relation to PA, AF, and energy intake (EI) in Greek schoolchildren, as they progressed from age 12 to 14 yr.

METHODS

The present study adopted a 2-yr design incorporating three data collection time points (i.e., baseline, first year, second year) and it is part of a large project on aspects of health and fitness in Greek schoolchildren. The baseline survey, which provided the basis for the current investigation, has been published elsewhere (6). Our aim was to examine the association between CHD risk factors (high-density lipoprotein ((HDL)-C, low-density lipoprotein (LDL)-C, HDL-C, triglycerides (TC), systolic and diastolic

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Submitted for publication January 2005.
Accepted for publication July 2005.

0195-9131/05/3712-2070/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/01.mss.0000178099.80388.15

blood pressure) and lifestyle parameters (fitness, fatness, fat intake, physical activity) in schoolchildren 12 yr of age.

Participants. At the baseline data collection time point (TP_1), 210 schoolboys and schoolgirls aged 12.3 yr volunteered. Participants were derived from all seven secondary schools in the provincial urban town of Katerini (~50,000 citizens), Greece (~10,000,000 citizens). This sample represented 29.4% of all schoolchildren 12 yr of age who were living in the typical middle-class Greek town of Katerini. During the second and third data collection time points, the sample size was 204 (TP_2) and 198 (TP_3), respectively. The dropout from TP_1 to TP_3 was 12 participants (eight boys and four girls), mainly because of illness and family relocation. Written informed consents, from both participants and their parents, were obtained after being given full oral and written explanation of the data collection procedures. The research ethics committee of the University of Wolverhampton, UK, approved the procedures and permission was granted from the Greek Ministry of Education.

Data collection. Participants were annually subjected to anthropometry, maturity status, Ad, PA, AF, and EI assessments. Identical protocols were used for each assessment conducted by the same trained investigators. All data were collected at the beginning (i.e., October) of three consecutive school years.

Anthropometry. Age (accurate to 1 month) was recorded. Standing height was measured to the nearest 0.5 cm using a Seca Stadiometer 208, with each subject's shoes off and head at the Frankfort horizontal plane. Body mass was assessed to the nearest 0.5 kg (Seca Beam Balance 710). Using an established equation (16), previously applied in Greek children (6), percent body fat was calculated from two skinfolds (i.e., triceps and medial calf, mean of two measurements) with a Harpenden (John Bull, England) caliper. Ad was defined as the total percent body fat reflecting the amount of fat stored in adipose tissue.

Maturity status. Sexual maturation (maturity status) was self-assessed by participants, using Tanner's criteria (23). Girls rated their breast development and boys their genital development from standard descriptions and pictures on scales from 1 (prepubertal) to 5 (postpubertal). Relevant information from each participant was collected by a pediatric medical doctor at the state hospital of Katerini, Greece. The chosen ratings were subsequently used for statistical analyses.

Physical activity. The Past-Year Physical Activity Recall Questionnaire (1) was used, wherein participants were encouraged to recall the total time spent during the past year on school physical education, organized sport, and other leisure-time activities. The calculated times were then combined with the metabolic cost of each activity (2) to estimate a total PA score expressed in kilocalories per kilogram per day. The questionnaire was administered in the participants' class rooms under the supervision and assistance of the principal investigator.

Aerobic fitness. The shuttle run test was used to assess AF. In brief, groups of 10 to 15 participants ran up and down a 20-m track at a steadily increasing pace ($0.5\ km \cdot h^{-1} \cdot min^{-1}$), controlled by signals from a standardized tape recording. Participants were instructed to keep pace with the signals for as long as possible. The test was terminated if the participant could not keep up the required running pace for two consecutive laps. The maximal speed ($km \cdot m^{-1}$) attained during the final stage of the test was subsequently used to calculate AF in $mL \cdot kg^{-1} \cdot min^{-1}$. The test was conducted indoors (in the schools' gymnasia) by the same investigator and an assistant who was responsible for recording the individual scores.

Energy intake. Information on EI was obtained by means of a 7-d dietary diary. A photographic album illustrating graduated portion sizes (1/1, 1/2, 1/4) of the most commonly consumed food items were used to assist keeping the diaries. Participants and their parents were instructed on how to keep a manual record of the amount and type of food consumed on seven consecutive days. They were given a booklet where each day was divided into five sections (i.e., early morning, midmorning, noon, afternoon, and evening), where they inserted the required information. Great care was taken to document the frequency, amount, and preparation methods of the consumed food items. After collection, each questionnaire was reviewed by the investigators to assure completeness. Using the Greek Food Composition Table (27), EI was estimated and expressed in kilocalories per kilogram per day.

Statistical analyses. Wilcoxon signed rank tests with a Bonferroni adjustment were used to compare the studied variables at different time points (TP_1 vs TP_2; TP_2 vs TP_3; TP_1 vs TP_3). For studying the dependent and independent variables, two further analyses were employed. The first (GEE_1) included calculations aiming to extract inherent longitudinal associations between the dependent and independent variables. To provide further evidence of association (by adopting a model suggesting that the independent variables should exist before the dependent variables in time (20)), the second analysis (GEE_2) used independent variables at TP_1 and TP_2 to predict the behavior of the dependent variable at TP_3. Because the data were collected across successive points in time, the GEE approach was employed for both GEE_1 and GEE_2 to account for subject- and item-specific dependency among the repeated observations. Using an extended approach for the mean structure of GEE, Fisher's z transformation was conducted to establish the functional relation between the correlation coefficients and the association parameters.

The GEE_1 analysis determined the longitudinal association between changes in Ad (dependent variable) and PA, AF, and EI (independent variables) throughout the study period. Covariates for gender, age, and maturation status were included in the model, whereas the data were grouped by time of measurement (i.e., TP_1, TP_2, TP_3). The analysis was repeated (gender not included as a covariate) with additional gender-specific classification to account for gender differences in the studied variables.

The GEE_2 used the mean PA, AF, and EI at TP_1 and TP_2 (independent variables) and Ad at TP_3 (dependent variable). Covariates for gender as well as age, maturation status, PA, AF, and EI at TP_3 were included in the model. The analysis

TABLE 1. Subject population numbers and selected parameters as assessed throughout the study (mean ± SD).

		TP_1	TP_2	TP_3
Participant Numbers	Entire sample	210	204	198
	Boys	117	112	109
	Girls	93	92	89
Age (yr)	Entire sample	12.3 ± 0.6	13.3 ± 0.6†	14.2 ± 0.5†‡
	Boys	12.3 ± 0.6	13.3 ± 0.6†	14.2 ± 0.5†‡
	Girls	12.3 ± 0.6	13.3 ± 0.6†	14.3 ± 0.5†‡
Mass (kg)	Entire sample	47.6 ± 9.9	53.0 ± 10.7†	58.6 ± 11.5†‡
	Boys	47.7 ± 9.9	54.4 ± 11.3*†	61.5 ± 11.9*†‡
	Girls	47.4 ± 9.8	51.3 ± 9.8*†	54.1 ± 9.1*†‡
BMI ($kg \cdot m^{-2}$)	Entire sample	20.0 ± 3.3	21.0 ± 3.5†	21.6 ± 3.3†‡
	Boys	20.1 ± 3.1	21.2 ± 3.5†	21.8 ± 3.1‡
	Girls	20.0 ± 3.5	20.8 ± 3.6	21.3 ± 3.2‡
Sexual maturation (Tanner stage)	Entire sample	3.1 ± 1.0	4.0 ± 0.8†	4.4 ± 0.7†‡
	Boys	3.0 ± 1.6	4.3 ± 0.7†	4.7 ± 0.5†‡
	Girls	3.1 ± 0.9	3.6 ± 0.6†	4.1 ± 0.7†‡
Fat-free mass (kg)	Entire sample	35.8 ± 5.4	40.7 ± 6.3†	44.6 ± 8.0†‡
	Boys	36.4 ± 5.6	42.5 ± 6.8*†	48.7 ± 7.7*†‡
	Girls	35.2 ± 5.0	38.4 ± 4.8*†	39.6 ± 4.8*†‡
Adiposity (%)	Entire sample	23.3 ± 9.1	22.1 ± 8.9†	22.3 ± 9.8‡
	Boys	22.3 ± 9.5	20.5 ± 9.3*	19.5 ± 9.9*‡
	Girls	24.4 ± 8.4	24.0 ± 7.8*	25.9 ± 7.7*
Physical activity ($kcal \cdot kg^{-1} \cdot d^{-1}$)	Entire sample	37.2 ± 5.1	34.1 ± 4.2†	33.6 ± 4.4‡
	Boys	39.2 ± 4.3*	36.2 ± 3.6*†	35.7 ± 3.9*‡
	Girls	34.8 ± 4.9*	31.7 ± 3.4*†	31.2 ± 3.9*‡
Aerobic fitness ($mL \cdot kg^{-1} \cdot min^{-1}$)	Entire sample	32.6 ± 6.2	34.8 ± 7.1†	36.0 ± 7.6†‡
	Boys	34.3 ± 6.6*	37.7 ± 7.1*†	39.6 ± 6.8*†‡
	Girls	30.5 ± 4.8*	31.2 ± 5.3*	30.9 ± 5.1*
Energy intake ($kcal \cdot kg^{-1} \cdot d^{-1}$)	Entire sample	54.9 ± 6.8	54.6 ± 6.0	57.2 ± 7.5†‡
	Boys	57.0 ± 5.7*	56.5 ± 4.8*	59.2 ± 7.4*†‡
	Girls	52.2 ± 7.1*	52.3 ± 6.5*	54.3 ± 6.2*†‡

TP_1, time point 1 (baseline); TP_2, time point 2 (first year); TP_3, time point 3 (second year); BMI, body mass index.
* Values significantly different ($P < 0.05$) between genders; † values significantly different ($P < 0.05$) from preceding time point; ‡ values significantly different ($P < 0.05$) between TP_1 and TP_3.

was repeated (gender not included as a covariate) with additional gender-specific classification to account for gender differences. All analyses were carried out with SAS (version 11.0, SAS Institute Inc., Carry, NC, US). The level of significance was set at $P < 0.05$.

RESULTS

Descriptive analyses. Descriptive data of the entire cohort appear in Table 1. Anthropometrical characteristics are in line with recently published epidemiologic data on Greek pediatric populations (15), suggesting that the present sample is representative of periadolescent Greek children. Most of the studied parameters demonstrated changes as subjects progressed from TP_1 to TP_3. At TP_1, for example, most of the subjects were at early- or midpuberty, whereas at TP_3, over 90% of the cohort reached late puberty. Ad decreased significantly ($P < 0.05$) from TP_1 to TP_3 in boys and demonstrated a nearly significant increase in girls ($P = 0.06$). Also, a significant increase was noted in EI ($P < 0.05$) with a concomitant decrease in PA ($P < 0.05$) for both genders, whereas boys further demonstrated a significant increase in AF ($P < 0.05$) as they progressed from age 12 to 14 yr.

Generalized estimating equations analyses. Table 2 depicts the results from GEE_1 and GEE_2 analyses employed to assess the longitudinal association, between changes in Ad and a group of independent variables (i.e., PA, AF, and EI). GEE_1 revealed that changes in Ad were significantly associated with PA ($\beta = -0.16$; $P < 0.001$) and AF ($\beta = -0.09$; $P < 0.05$) for all participants. This was not the case, however, for EI ($\beta = 0.04$; $P > 0.05$). The same pattern was retained following gender-specific classification, despite that Ad decreased in boys and increased in girls. The GEE_2 revealed that the main influential factor during TP_1 and TP_2 for the development of Ad at TP_3 was PA ($\beta = -0.14$; $P < 0.001$), followed by AF ($\beta = -0.10$; $P < 0.05$), whereas EI was not a significant factor ($\beta = 0.02$; $P > 0.05$). This was valid even after controlling for age, maturation status, PA, AF, and EI at TP_3. Again, the same pattern was retained following gender-specific classification.

DISCUSSION

This study examined changes in Ad in relation to PA levels, AF, and EI in Greek schoolchildren as they progressed from age 12 to 14 yr. At the end of the study, all four

TABLE 2. Generalized estimating equations (GEE) standardized (β) regression coefficients (95% confidence intervals) for the first (GEE_1) and second (GEE_2) analyses of association between Ad and the independent variables PA, AF, and EI.

			Grouped Data[b]	
		All Participants[a]	Boys	Girls
GEE_1	PA	−0.16 (−0.20 to 0.10)**	−0.17 (−0.22 to 0.09)**	−0.15 (−0.19 to 0.07)**
	AF	−0.09 (−0.16 to 0.02)*	−0.10 (−0.18 to 0.03)*	−0.08 (−0.15 to 0.01)*
	EI	0.04 (−0.03 to 0.08)	0.03 (−0.03 to 0.07)	0.04 (−0.05 to 0.09)
GEE_2	PA	−0.14 (−0.18 to 0.09)**	−0.14 (−0.18 to 0.09)**	−0.14 (−0.19 to 0.09)**
	AF	−0.10 (−0.15 to 0.03)*	−0.10 (−0.15 to 0.03)*	−0.10 (−0.16 to 0.03)*
	EI	0.02 (−0.03 to 0.06)	0.02 (−0.02 to 0.06)	0.03 (−0.03 to 0.07)

PA, physical activity ($kcal \cdot kg^{-1} \cdot d^{-1}$); AF, aerobic fitness ($mL \cdot kg^{-1} \cdot min^{-1}$); EI, energy intake ($kcal \cdot kg^{-1} \cdot d^{-1}$).
* $P < 0.05$; ** $P < 0.001$.
[a] Corrected for gender, age, and sexual maturation.
[b] Corrected for age and sexual maturation.

parameters demonstrated significant changes compared with baseline levels. To our knowledge, no published longitudinal data involving Greek schoolchildren exist for direct comparisons. The present results, however, are in line with previous cross-sectional epidemiologic studies, suggesting that Greek pediatric populations demonstrate an increased prevalence of obesity (15), decreased PA levels (18), low AF levels (14), but similar EI (26), compared with age-related peers from other countries. It could be argued, therefore, that Ad is linked to a low PA and AF profile exhibited by the present schoolchildren.

The advantage of using the GEE analysis in the present study stems from the clustered nature of our data and the need to isolate the effect of each independent variable. The GEE analysis does not require a specific assumption of the observations' joint distribution, and is advantageous in that it yields consistent regression coefficients estimates and their variances even in cases when the correlation-structure of the dataset is erroneously specified. Although this is an important strength of the GEE analysis, it, however, also limits the interpretation of the results in such a way that the separation between within-person effects (longitudinal) and between-person effects (cross-sectional) cannot be made. Another problem in investigating longitudinal relationships among Ad, PA, AF, and EI is to establish causality, because the underlined mechanisms implicated are complex (28). According to deterministic models of causality, "diseases have causes and if these causes are present improper time windows, diseases will follow" (20). Using this premise, although the present design cannot fully establish causality, the present results demonstrated very strong longitudinal associations because the independent variables existed before the dependent variable in time, while controlling for subject- and item-specific dependency among the repeated observations. Because Ad, PA, AF, and EI can be confounded by factors associated with children's growth and development (17), the current data were corrected not only for age and gender but also for maturation stage.

The present GEE analyses revealed a strong longitudinal association between PA levels and Ad in Greek children, compared with AF or EI. These findings are in line with longitudinal data from other European (22) and North American (12) countries showing Ad to be inversely related to PA and AF levels. In contrast, our results challenge the findings of Goran et al. (9) who observed no longitudinal associations between PA and Ad in a sample of preadolescent children. These authors found that the main predictors of changes in body fat were sex, initial fatness, and parental fatness, but not PA. This discrepancy could be attributed—*inter alia*—to the fact that they used younger participants than those in the present study (e.g., older children have better movement efficiency (8)), and adopted a more rigorous assessment of PA, but only for 14 d, which may have been confounded by seasonal variations in PA. Obtaining valid and reliable measures of PA in children, in general, may also account for the observed discrepancy (28). Nevertheless, direct comparisons with other studies are difficult owing to variations in methodology, subject age, and ethnicity (7,12).

Physical activity is hypothesized to protect from the development of obesity through two main channels. First, through increased energy expenditure, mainly because of the high cost of activity itself, which normally appears with concomitant increases in resting metabolic rate (RMR) (21). This limits the likelihood of positive energy balance. Second, PA has beneficial effects on substrate metabolism, with an increased reliance on fat, relative to carbohydrate, for muscular fuel utilization (3). In general, the effect of PA on caloric expenditure is accumulative and, with time, small amounts of activity can add up to significant weight loss. In children, however, aspects related to growth and development affect fat and weight regulation in different ways in boys and girls (13). Unlike males, adequate body fat in young females is necessary for menarche to occur and this can be partly achieved through reduced PA levels. Indeed, a 50% reduction in PA has been observed before female puberty (10), indicating the existence of a biologically regulated, energy-conserving mechanism in favor of fat accumulation. This hypothesis fits well with the current results, where girls were found to be less active than boys.

We also found that our participants exhibited lower AF levels compared with age-related peers from other countries (15,18,26), and that AF was associated with longitudinal changes in Ad. The latter can be explained by the fact that low AF reduces muscular fat oxidation capacity, which may decrease the tolerance of dietary fat and increase body weight and, therefore, Ad (7). We further found, however, that EI was not significantly associated with Ad. Studies of the role of EI in the etiology of obesity in childhood are limited and equivocal (4). Methodological problems (e.g., underestimation of nutritional intakes), especially from obese individuals, may have confounded the results. Another explanation for the observed lack of association between EI and Ad may rest on the fact that, unlike Ad, PA, and AF, the present subjects demonstrated similar EI levels to those of age-related peers from other countries (26).

It is reasonable to assume that the present results may have been influenced by methodological limitations such as seasonality in AF and EI patterns, because the data were collected at a specific month of the year. To the authors' knowledge, a significant seasonal variation has only been confirmed for PA, whereas no convincing evidence exists to support the equivalent for AF and EI. The effect of seasonality in PA was accounted for by using the past-year physical activity recall questionnaire, which provides information for the entire previous year, but the questionnaire has not been fully validated in Greek adolescents. A possible underestimation of nutritional intakes, especially from obese individuals, and the role of genetic factors in susceptibility to dietary changes may be also reflected in our results (26). We attempted to control these inherent dietary-monitoring limitations by instructing participants and their parents on how to keep the dietary record based on the amount and type of food consumed. The boys' slightly high mean sexual maturation values suggest either an early maturation of this cohort, or an overestimation of their pubertal status (which is typical for adolescent males). Further, we were unable to examine fully the causation of the various phenomena in our study because of the observational nature of the adopted longitudinal design. Nevertheless, our analyses represent strong longitudinal evidence because the independent variables existed before the dependent variable in time. Because the relationships among Ad, PA, AF, and EI are complex (28), however, future studies adopting longitudinal-interventional designs will provide a more comprehensive assessment of causality among these parameters. Finally, our results should be interpreted with some caution because they are based on a relatively small number of participants (range, 198–210).

In conclusion, and within study's limitations, the present longitudinal data demonstrated that changes in physical activity and, to a lesser extent, aerobic fitness predict changes in adiposity in Greek schoolchildren. Promotion of PA should receive special attention in the design of health programs aiming at preventing or reversing undue weight gains, which should begin early in life.

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